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TUMOR NECROSIS FACTOR IN THE SERA OF MYCOBACTERIUM BOVIS PPD REACTOR AND NON-REACTOR BUFFALOES

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ABSTRACT: Nine buffaloes were screened for the presence of cytotoxic factors in their sera using tumor necrosis factor susceptible - L 929 cell line. Health status of the animals and the sensitivity to tuberculin were also recorded. Two animals were found positive for the presence of cytotoxicity, of these, one was reactive to the tuberculin skin testing and the other one was non-reactive, though very much debilitated.

Tumor necrosis factor (TNF) was first identified in the serum of mice following endotoxin injection (Carswell *et al.* 1975). It was also considered as 'cachectin' for its role in inducing cachexia which resulted in a combination of debilitating responses including weight loss, negative nitrogen balance, often with anorexia (Clemens, 1991).

Circulating TNF was detected in the serum of human and animals in many types of diseases. It was found in serum of cancer patients (Balkwell *et al.*, 1987) and patients suffering from systemic lupus erythematosus (Maury and Teppo, 1989). Patients suffering from severe infectious purpura (Girardian *et al*, 1988) and fatal meningococcal meningitis had high level of TNF in the sera (Waage *et. al*, 1987).

The present study was conducted on buffaloes to study the positive correlation of tuberculin skin reactivity and serum cytotoxicity on tumor necrosis factor-susceptible L 929 cell line.

MATERIALS AND METHODS

Animals and TNF susceptible cells: The buffaloes for the cytotoxicity screening were obtained from Livestock Production Research Section, IVRI, Izatnagar. The TNF susceptible I. 929 cell line was supplied by National Facility for Animal Tissue and Cell Culture, Pune.

Collection of serum and tuberculin test: Prior to the study, general physical examination of the selected buffaloes was done. Bovine tuberculin (PPD) obtained from Biological Products Division, IVRI, Izatnagar was injected intradermally (0.l ml) along with the control (sterile NSS). The skin thickness of both the area injected with PPD and NSS, was measured at 72 hrs. Ten ml blood was taken aseptically from jugular vein of each buffalo before PPD testing and serum separated, centrifuged and stored at - 20°C until assayed.

Assay of serum cytotoxicity activity: The cytotoxic activity of the sera samples was measured on L 929 cells with actinomycin D (Sigma) following the procedure of Ohno *et al* (1990) with modifications. Briefly, L 929 cells were seeded at a density of 4 x 10^4 per well of 96 well plastic tissue culture plates (Nunclon, Denmark) in 100 ul of RPMI1640 growth medium. After incubation for 24 hrs., the medium was discarded and replaced with two-fold serial dilutions of the test sera samples containing 4 μg/ml of actinomycin D in RPMI 1640 containing 2% of new-born calf serum (NBCS). Three wells were added with only growth medium and another three with RPMI 1640 medium having 5% of Tritox X-100 (Sigma) for 0% and 100% cell lysis (as controls) respectively.

After an incubation of 18 hrs., the culture medium was discarded and the L 929 cells were further incubated with RPMI 1640 containing 5% NBCS and 0.006% neutral red (Sigma) for 75 min. After washing with 0.085 M phosphate buffered saline (PBS, pH 7.2), neutral red in the viable L 929 cells was eluted into 100 μl of 0.01 N HC1 containing 30% ethanol and the absorbance of each of the wells was read at 490 nm in ELISA reader (Molecular Device, U.S.A.). The cytotoxicity was calculated using the following formula:

$$\text{Cytotoxicity (\%)} = \frac{A(O) - A(S)}{A(0) - A(100)} \times 100$$

Where, A (0) = Absorbance in the well with 0% lysis of the cells
A (I00) = Absorbance in the well at 100% lysis of the cells
A (S) = Absorbance in the well added with serum sample.

RESULTS AND DISCUSSION

The cytotoxicity showed by the buffalo serum might have some correlation with the health condition of the animals and the result of tuberculin test done on them. Sera from two animals (serial number 2 and 8) showed a very low percentage of cytotoxicity and were negative to tuberculin test (Table 1 and 2). Animal number 1, apparently normal neither showed skin reactivity to PPD nor presence of cytotoxic factor (s) in the serum (Table 1 and 2). Two animals (number 4 and 6) showed mild skin reactivity to PPD and also cytotoxicity in their sera (number 4 animal cytotoxicity at 1/64 dilution, 30.22+1.73). Sera samples of two animals number 3 and 7 showed the highest cytotoxicity among sera of animals tested, of which animal number 7 was most reactive to tuberculin (Table 1 and 2).

Cattle showed non-responsiveness to PPD in an advanced stage of tuberculosis (Plackett *et. al.*, 1989). Animals (number 5 and 9) apparently normal did not show skin reactivity to PPD but had some cytotoxicity in their sera.

TNF activity was often regarded as being synonymous with cytotoxicity for sensitized target cells. It could be argued that serum samples from animals might contain other toxic factors that also could kill cells in the TNF bioassay. Lymphotoxin, interleukin 1 and interferons, all caused cytostasis in some tumor cell lines *in vitro* (Lachman *el al,*, 1986). However, Mackay *el al.* (1991) have reported that the antibody raised against human TNF was able to remove more than 90% cytotoxicity from the endotoxin treated horse serum. In the present study increased cytotoxicity observed after dilution of serum of certain animals might be due to presence of some inhibitory factor (s) which get

diluted. Infact, human urine and serum particularly in pathological conditions might contain protein which could interfere with the functions of TNF when tested on susceptible cell lines *in vitro* (Seckinger *el al.,* 1990).

Table I. Report of the physical examination and the result of tuberculin testing of the buffaloes screened for serum cytotoxicity.

Sl.. No. of animals	**Description/** health status	**Result of tuberculin** tcst(cm) (control/PPD)	**+/–**
1.	Apparently normal	1.6/1.8	–
2.	Apparently normal	1.7/1.9	–
3.	Weak and hide bound	1.6/1.7	–
4.	Weak	1.7/2.4	±
5.	Apparently normal	1.3/1.6	–
6.	Weak	1.7/2.9	±
7.	Weak	1.5/3.5	+
8.	Weak, diarrhoeic	–	–
9.	Apparently normal	1.5/1.7	–

Table 2. Cytotoxicity of sera samples of the screened buffaloes.

(% Cytotoxicity / Dilutions of the sera samples)

Serial no. of animals	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/64**
1.	0.00	0.00	0.00	0.00	N.D.	I6.94±1.61
2.	9.22±0.93	2.44±1.19	01.69±1.38	2.07±1.69	7.37±2.34	N.D.
3.	84.55+2.01	55.74±1.51	71.38±11.59	31.93±2.89	38.4±2.60	50.39±7.2l
4.	4.39±2.69	11.35±1.80	13.60±1.79	13.28±6.41	12.32±1.70	30.22±l.73
5.	25.29±4.39	21.64±1.55	23.36±1.43	17.57±2.21	23.89±1.80	23.36±1.32
6.	35.14±1.06	30.32±0.76	30.86±7.46	46.51±2.80	40.94±0.46	30.33±2.32
7.	73.62±0.69	50.47±0.40	53.05±0.26	46.29±4.06	39.12±16.40	21.73±3.99
8.	N.D.	8.52±3.48	5.79±3.76	12.87±4.42	10.95±6.54	I0.46±2.45
9.	25.11 ±3.94	9.33±3.97	4.50±2.05	20.44±3.92	28.50±2.05	15.61±4.01

Values indicate the average of three tests ± S.E.: N.D. = Not Done

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